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Developmental Studies of Wheat in Microgravity

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Some history of the project

This project has had a rather complex history. It began with an *Announcement of Opportunity* in 1984, which solicited proposals for flight experiments related to Gravitational Biology or to the NASA CELSS program—at least our proposal was aimed at a combination of those two NASA programs. We proposed a series of experiments with a super-dwarf cultivar of wheat in the so-called *Plant Growth Chamber* (PGC), designed for a mid-deck locker in the U.S. Shuttle. Evaluation of the proposal was delayed by the Challenger accident, but we were informed in 1987 that our proposal had been "accepted for definition studies," but that funds were limited and would not be forthcoming at that time. By 1990 we had not received funding from the NASA Flight Program that originally distributed the AO84 although I had been funded for basic studies on plant mechanical stresses and gravitropism through the NASA Gravitational Biology Program (Thora Halstead, Director) and for studies on growth of wheat in controlled environments through the NASA CELSS Program (James Bredt, then Maurice Averner, Directors).

In late 1989 and early 1990, Halstead and Averner agreed to fund some preliminary studies aimed at preparations for the flight experiments we had outlined in the AO84 proposal. This funding went through the Kennedy Space Center, with **William M. Knot** as the NASA Technical Officer. We worked with various people at the Kennedy Space Center, often thinking that we might be within months of a flight experiment, but this never materialized. Various technical problems seemed to halt the efforts. There were attempts to construct a new *Plant Growth Facility* (e.g., by William Scheld in College Station, Texas), for example, and we proposed various ways to increase the irradiance levels in the existing or in a new plant growth chamber. We wanted to replace the small, plastic *Plant Growth Units*, as they were called, but this met some opposition. **Gail Bingham**, a member of the Plants, Soils, and Biometeorology Department and also the Space Dynamics Laboratory at Utah State University, became a very active participant in the project, working on design of an infra-red CO_2 analyzer that was to be a unique part of our project.

In the meantime, at a conference in Krasnoyarsk, Siberian Russia, I met two Russian scientists, Dr. Alexander Mashinsky and Dr. Galena Nechitailo, who invited me (and also Dr. Ted Tibbitts at the University of Wisconsin and Dr. Cary Mitchell at Purdue University) to fly our super-dwarf wheat (or other crops) on the Russian Space Station *Mir*. There were several visits from these and other Russian scientists to Utah State University, and I visited the Institute of Biomedical Problems in Moscow in July and again in November, 1992. We were planning both U.S. Shuttle and *Mir* experiments at the same time and with support of the project of which this is a final report. It appeared, however, that the Shuttle experiments were less likely to fly. There was not enough financial support for the hardware modifications that would have to be made. Besides, our proposed experiments could be only marginally successful when limited by the short duration of Shuttle flights, but *Mir* would offer much longer durations. As our activities with the Russians increased, we were told that we were to work with personnel at NASA Ames rather than the Kennedy Space Center (where emphasis was on Shuttle experiments). Eventually, our contacts at Kennedy Space Center dropped to almost nothing.

The November visit to Moscow included six members of our Utah team plus David Bubenheim of NASA Ames. During the visit, there was much talk about a Shuttle flight to *Mir*, and shortly after we returned, the U.S. and Russia agreed to such a program (called **Spacelab Mir-1**). Our project was accepted as part of this program, and we finally began to receive the levels of funding necessary for a project that involved considerable hardware development and a complex interaction with Russian scientists. To speed matters, however, the funding continued to come through the Kennedy Space Center as part of the project being reported here. This approach was finally terminated with the beginning of fiscal 1994, and the funding began to be sent through the NASA Ames Research Center. There was some overlap as the project being reported here continued until Feb. 28, 1994. The remainder of this report describes our activities, mostly since we became part of Spacelab Mir-1. Most of the funding came through the Kennedy Space Center except for the most recent months, which were funded through the NASA Ames Research Center.

Description of the *Mir* experiment (Greenhouse 2)

We are now cooperating with scientists at the Institute of Biomedical Problems in Moscow, notably **Yuri E. Sinyak** (Head of Department), **Vladimir N. Sychev** (Head of Laboratory), **Margarita Levinskikh** (Principal Investigator), **Yuli Berkovitch** (engineer in charge of *Svet*, the Russian plant growth chamber), and **Igor G. Podolsky** (specialist on the substrate). The Russian scientists are carrying out preparatory ground studies and will also carry out ground controls; these will be duplicated with modifications at Utah State University and at the NASA Ames Research Center, where we are cooperating with **David Bubenheim** (plant physiologist) and **Boris Yendler** (who cooperates with Podolsky in Moscow and **Scott Jones** at Utah State University on studies of the status of water in the substrate).

I am principal investigator for the U.S. team, and instrumentation (substrate moisture and gas exchange measurements) is being developed under the direction of **Gail Bingham**. This hardware development is taking place in the Space Dynamics Laboratory at Utah State University. Functional prototypes of all the instruments shortly to be described exist, and final instrumentation is to be delivered to Moscow by July 1, 1994.

After the necessary flight qualification and astronaut/cosmonaut training, this equipment will be transported to *Mir* (on Russian transport vehicles) near the end of the year and installed in *Svet*, which is already on *Mir* (including its substrate module). Installation of the equipment is to occur in early January 1995, at which time a seed-to-seed experiment will be initiated. Measurements of plant gas exchange rates (transpiration and photosynthesis) will be followed continually, and summary data will be down linked on a daily basis. This information will be critical to proper irrigation management in the young crops. Respiration levels will be measured during 4-hour dark periods on a weekly basis.

If the plants grow well enough, they will be sampled and fixed at five times during the seed-to-seed experiment, and mature plants will be harvested:

1. Young vegetative seedlings (six days after planting),
2. Early-floral-stage seedlings (14 days),
3. Boot stage (heads surrounded by leaf; ca. 35 days),
4. Anthesis (appearance of pollen; ca. 48 days), and
5. Seed development stage (ca. 62 days).
6. Harvest of mature plants (ca. 90 days).

The first two samplings will be done at arbitrary dates; the other times will depend on plant development (if astronaut/cosmonaut time can be adjusted). Fixed samples will be returned to earth for microscopic examination. Leaf measurements will be made at each sampling time to scale the calculations of photosynthetic and respiratory rates. Excess biomass used in leaf measurements will be dried in bags with desiccant and stored along with fixed tissue. When plants are mature (nearly dry), they will be harvested and stored with enough desiccant to prevent growth of molds. Russian collaborators will study micro-organisms associated with the plants. Plants will be photographed at least at weekly intervals and at each sampling interval. After mature plants have been harvested, a fresh crop will be planted. Based upon experience during the seed-to-seed experiment, it is hoped that plants in the second planting will have reached a stage approximately equivalent to that obtained on earth after about 30 to 35 days. At this time the U.S. Shuttle will dock with *Mir*, and plants will be harvested and frozen in the GN_2 freezer brought up on the Shuttle. These plants will be analyzed on earth for a spectrum of plant hormones, carbohydrate reserves, free amino acids, minerals, and stress proteins; such analysis will provide insight into stresses experienced by the plants.

Svet has approximately 0.1 m^2 of plant growing area (about 28 x 28 cm). Irradiance is from a bank of small florescent tubes that provide 30 to 40 W m^{-2} (PAR = photosynthetically active radiation), which is equivalent to about 135 to 175 μmol m^{-2} s^{-1} (PPF = photosynthetic photon flux). There is no active cooling or heating, but cabin air is drawn over the lights and separately over the plants, keeping them fairly close to cabin temperature (sometimes slightly cooler than cabin temperature because of transpiration).

The root module is divided into two compartments (cassettes). Each compartment has two folds of fabric in which the seeds are planted (two rows of seeds for each cassette; actually, in a prepared holder brought to *Mir* from Earth). A moisture sensor is located between the two layers (rows) of folded fabric, and this fabric extends at the top down into the *balkanine* (ion-exchange material that acts as substrate for the plants) to the location of a moisture sensor. Water is added at the bottom of the folds of fabric on either side of the moisture sensor and a few centimeters (of *balkanine*) away from the sensor. Water can move up the fold and down to the sensor by capillarity without entering into the *balkanine*. In previous experiments with *Svet* on *Mir*, it appeared that the moisture sensor was giving readings of saturation although the *balkanine* may have been dry. Hence, the Russian scientists decided to cut the fabric (the wick) between the points where water is introduced and the moisture sensor. This they did in one of the two soil cassettes but not in the other, which was left as a control. These casssssettes have already been sent to *Mir.*

The instrumentation being developed at Utah State University begins by lining *Svet* with highly reflective material that increases the light reaching the plants by 20 to 30%. Two transparent bags will be placed over the plants, one above each root cassette (Fig. 1). Two bags are necessary because of the difference in the root modules (i.e., the wick being cut in one module). Measured volumes of air will be introduced through a manifold close to the plants. CO_2 and H_2O vapor will be measured by one sensor as the air enters the plastic bags and by another sensor in the airstream exiting the bags; differences will provide a measure of photosynthesis, respiration (during darkness), and transpiration. Eight moisture sensors (4 short and 4 long) will be inserted into each of the two compartments of the root cassette, these being placed at various strategic locations so that moisture levels near the roots, in the fabric, and in the *balkanine* can be monitored with some care. Another instrument cluster will be located on the upper end of a rod in each bag. These sensors will detect leaf temperature (IR thermometer), air temperature, and irradiance. Cabin O_2, pressure, and temperature will be measured in the air stream entering the bags.

Detailed sampling and harvesting procedures have been worked out with crew trainers at NASA Ames, with a Russian trainer visiting at Johnson Space Center, and with cosmonauts and others in Moscow. These procedures are somewhat complex because they require raising of the plastic bags, removal of the plants with special instruments, and placing of the sample tissue into triply contained fixative bags, this taking place within a glove bag. Special color charts have been developed to record the color status of the plants. Techniques of photography are still being developed.

Another complication that could influence the results is the growth of molds and other microflora. Such growth has been observed in a few ground studies. Our Russian colleagues (especially **Dr. Lola Chernova**, a microbiologist active in the Russian Space Program) are anxious to examine and sample the root module for its microflora immediately after it is returned to earth, and we may add a microbiologist to our U.S. team.

Work in progress: Misgivings about the experiment

As implied above, substrate conditions probably limited growth in previous experiments in *Svet*. This was not known for certain because the substrate container was not returned to earth but jetessoned in space. Cutting the wick in one compartment of the soil module is the only modification made for our experiment. This will not be enough to solve the problem. Studies of Yendler at Ames, and Podolsky at the Institute of Biomedical Problems, suggest that water may not even get out of the wicks into the substrate. Our measurements of moisture-release curves of the *balkanine* show that it is more hydrophobic than might be desirable and that plants will be able to obtain moisture only over a relatively narrow range of moisture levels within the *balkanine*. Thus it is probable that the plants in our experiment will not have adequate moisture or especially adequate nutrient elements.

Svet Instrumentation System

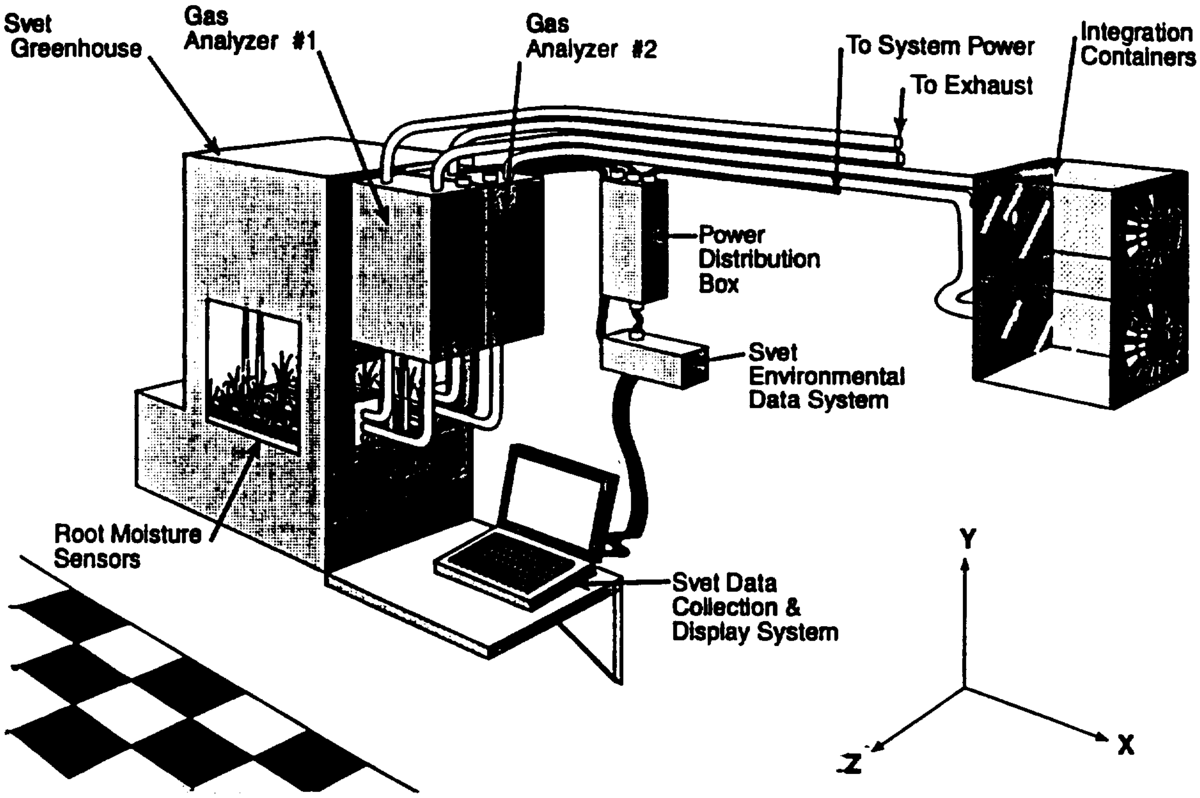

Figure 1. The *Svet* instrumentation system. Note the two gas analyzers connected to two transparent, plastic bags, one over each root cassette.

Our many moisture sensors (compared with the previous single sensor) should be of great value in detecting the moisture status in the soil module and indirectly suggesting the nutrient status (i.e., if water and roots do not penetrate into the balkanine). We will analyze returned samples (dry biomass) for nutrient elements, and results of these analysis will also be valuable in interpreting plant responses in this experiment. We hope that the root module will be returned to earth this time.

CO_2 levels won't be *controlled* although they will be *measured* with accuracy enough to calculate photosynthetic rates. Air going into *Svet* will be filtered to remove particulates, but this filtering will be minimal—no scrubbing of ethylene, for example.

Irradiance will be improved over previous experiments thanks to the reflective lining and use of a light module with fresh fluorescent tubes, probably enough to produce relatively good plant growth. We would expect better growth if light levels were higher.

It seems possible to the members of our current team that these deficiencies will prevent us from obtaining the most desired results; that is, plants that are not limited in their growth by the usual environmental parameters but are subject only to the stresses of microgravity and possibly ionizing radiation unique to the space environment. The monitoring that we will have in this experiment should allow a much better interpretation than has been possible before. We hope to continue our experimentation after this experiment is complete, attempting to rectify known adverse factors and any others that become apparent during our present study. We have applied for funture funding.

Work in progress: Ground studies

The prototype instrumentation is being set up in Moscow at the Institute of Biomedical Problems with a ground version of *Svet* that exists there (May, 1994). Seeds will be planted, and Superdwarf wheat will be grown to maturity, hopefully at least twice before the final instrumentation is transported to *Mir*. Construction of two flight-like *Svet* growth chambers has been commissioned by NASA at the Bulgarian institute that originally designed and constructed the existing models of *Svet*. Instrumentation will be added to at least one of these at Utah State University for use in syncronous ground control tests. In addition, we are preparing twelve *Svet* mockups in which we will carry out experiments between now and post-flight analysis. The first experiment will have six of the chambers at 23°C and the other six at 27°C. At each temperature condition, pairs of chambers will be held at 18, 21, and 24-hour photoperiods. Since each chamber has two bags, it will be possible to have four CO_2 concentrations at each photoperiod and temperature: 360 (ambient), 1200, 3500, and 7000 μmol mol^{-1} (equals ppm). It is important to study photoperiod because longer days result in more rapid development, but at least a four-hour dark period is desirable to allow study of respiration. (Only a six-hour dark period can be programmed on *Svet*, however.) Our ground experiments will determine whether this dark period can be a routine part of the schedule or only an occasional, manually controlled exercise to study respiration. We plan to have similar substrate conditions in all root modules during this experiment, but water stress and possibly changes in nutrient levels can be a part of future experimentation with this facility. It might also be desirable to allow temperature and even CO_2 concentration to vary during the experiment as will occur in *Mir*. We will keep this facility in active operation until well after the post-flight analysis and hopefully into the period of future experiments. (Development of such a facility has been an important goal for the past few years during the period being reported here.)

Plant hormones will be analyzed in frozen samples returned to earth. This will be by **John Carman** and a graduate student, **Rubin Nan**, with help from a technician, **Pamela Hole**, all at Utah State University. The techniques have been worked out in Carman's lab over the past few years and are being adapted to the current experiment. Carman will also measure water-soluble carbohydrates, free amino acids, and minerals. **William Campbell** and a graduate student, **Liming Jiang**, also at Utah State University, are practicing techniques of anatomical and histological analysis, also with help from Pamela Hole. Again, such techniques have been standardized for light and electron microscopy; these are being adapted for the present experiment. A special problem concerns the length of time during which samples must be stored in fixative at room temperature before return to earth.

David Bubenheim and his postdoctoral fellow **Mark Patterson** at NASA Ames are also studying plant growth under conditions representing those that may be encountered in *Svet* on *Mir*. These studies include growth of plants at different atmospheric pressures as well as CO_2 levels, and this group is developing techniques of anatomical and chemical analysis including mineral and protein analyses.

Yendler at NASA Ames, working in rather close conjunction with Podolsky at the Institute of Biomedical Problems and Jones at Utah State University, is studying movement of fluids in porous media on the ground and in space. Yendler and Podolsky have had a number of flight experiments on *Mir* and other Russian vehicles. Their results helped us to understand why an even distribution of moisture in a granular substrate is so difficult to achieve in microgravity.

An overriding aspect of the ground studies has been the hardware development going on at the Space Dynamics at Utah State University. A team of about 20 people, including electrical and mechanical engineers, have been intensively designing and constructing the instrumentation described above. As noted, functioning prototypes have been developed and are being used in ground studies, and final flight instrumentation is to be delivered by July 1, 1994.

Many of our ground studies from 1990 to 1993 examined a range of irradiance levels (Salisbury and Gillespie, 1992; Salisbury et al., 1993). These studies were carried out with the help of my technician, **Linda Gillespie**. Perhaps the most significant finding was that wheat plants will grow and yield at light levels so low that they approach the photosynthesis compensation irradiance where photosynthesis and respiration are equal, but that such low light levels greatly retard development so that much more time is required to produce mature plants. That is, under the low-light plants simply take longer to accumulate the photon energy that is essential to mature the vegetative and reproductive parts of the plant. (Close to and below the compensation point, this is simply impossible, and plants, although they may stay alive, typically fail to produce seed and only produce a very limited number of leaves.) These results suggest that recorded irradiance levels from Soviet or U.S. space experiments with plants were barely above the compensation point. That is, although plants grown under those irradiances might appear green and healthy, plants under higher irradiances grow much better. In most of the space experiments, only the low irradiances have been used in ground controls.

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